

The University of Chicago

The Normal Seasonal Reproductive Cycle in
the Male *Eumeces Fasciatus* Together
with Some Observations on the Effects
of Castration and Hormone
Administration

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDI-
DACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

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IN THE MALE EUMECES FASCIATUS TOGETHER
WITH SOME OBSERVATIONS ON THE
EFFECTS OF CASTRATION AND
HORMONE ADMINISTRATION ¹

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TWO PLATES (NINE FIGURES)

INTRODUCTION

The classical work of Lillie ('17) directed to the study of basic problems in the biology of sex has been followed by an ever increasing number of contributions to the knowledge of these broad problems. Whereas the greater number of studies has been conducted with the use of mammalian forms, both birds and amphibians have been abundantly used; relatively few investigations have been carried out on fish or reptiles. The great neglect of reptiles as experimental materials for such studies was pointed out by Moore ('32) in the first edition of *Sex and Internal Secretions*.

Attracted to the opportunities presented by reptilian materials, the writer has attempted an investigation conceived to encompass many of the fundamental aspects of sexual conditions and reproductive states in one of our common American lizards. The realization that a solid framework of basic information was required before experimental analyses could be intelligently applied caused the approach to be directed to a study of the normal seasonal changes occurring in such animals. The results of such a study are embodied in this

¹ This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

report, together with some information gained by employing experimental technics to further the analysis. From these initial studies many separate aspects have emerged that are receiving attention, but discussions of these are reserved for future publication.

MATERIAL AND PROCEDURES

The selection of the red-headed skink, *Eumeces fasciatus*, as the experimental animal is well-justified since the species is more widely distributed than other members of its genus, and since it approaches a type for its genus (Taylor, '35). The principal objection to it is the difficulty of obtaining specimens in numbers. While a few animals were collected in central Indiana, the majority came from supply houses that drew from the states of Arkansas, Florida, Missouri, and Tennessee. Quite nervous and wild when first caught or received, they readily acclimate to captive conditions. Since they are secretive by habit (Noble and Bradley, '33), means of seclusion was provided in the cages, and the routine included constant provision of water and feeding once to three times weekly, mealworms forming the basis of the diet.

This is a "medium sized species" according to Taylor ('35). The male animals reported on here measured, from the tip of the snout to the groin, around 60 mm., and weighed on the average, 10 gm. In the diagrammatic male the ventral surface is gray, the rest of the body a dull reddish or copper color which becomes bright red in the region of the head and neck. The body is stocky and has a muscular appearance, the angle of the jaws is wider than the neck. In the diagrammatic female there is no such difference between the jaws and the neck, the body is less stocky in appearance, the ventral surface is gray, while on the sides and back there is a ground color of light brown or gray upon which appear five longitudinal lines, dirty white in color. Both of these sexually dimorphic patterns develop out of the juvenile pattern of dark brown to black ground color with five stripes of bright yellow; one median and dorsal, a pair located laterally, and a pair dorso-

laterally. From a point near the hind legs to its tip, the tail is a vivid blue. Sexual maturity is probably reached before the dimorphic color changes are completed, since adults with somewhat confusing patterns are often encountered, thus rendering color an unreliable indication of sex in many cases.

The results presented below are based on study of a total of fifty-three adult male skinks, thirty-six of which were utilized as normal animals, and seventeen of which were used experimentally. A definite code in removing toes enabled the keeping of individual records; operations were accomplished by a single ventral incision, and separate sewing of the musculo-peritoneal and skin layers with fine silk was practiced. Fixation in Bouin's fluid, paraffin embedding, section at 8 micra, staining in Heidenhain's iron haematoxylin, and mounting in balsam or Clarite were the standard histological procedures, although other methods were used in a few instances. In injections, sites included the two thighs and the base of the tail.

It is a pleasure to acknowledge indebtedness to Dr. Carl R. Moore for suggestions and guidance during the course of this study; to Mr. Karl P. Schmidt of the Field Museum of Natural History for aid in taxonomy; and to Dr. Gregory Stragnell of the Schering Corporation for making available the hormones employed. A more extensive account based on these data has been previously rendered (Reynolds, '41).

REPRODUCTION IN THE NORMAL MALE EUMECES FASCIATUS

Below, a descriptive account of the morphology involved is followed by a description of the seasonal variations in the testis, the epididymis, and the metanephric kidney. The data are based on study of testes from thirty animals, epididymides from twenty-eight, and kidneys from twenty, with the seasonal sampling representing 10 months of the year. Where quantitative measures are used, the means are based on from ten to fifty measures per organ.

A. General morphology of the reproductive tract

When a "typical" testis-epididymal morphological pattern is envisaged, it is usually that exhibited by common mammals. Testicular lobules are separated by septulae testis; each lobule contains tubuli contorti converging into tubuli recti which in turn converge into an interlacing network of small tubules, the rete testis, located in the mediastinum. From here, testis products leave the testis by way of a number of small vasa efferentia which lead to a larger and very elongate tube, the ductus epididymis, which is convoluted into a small compact organ, the epididymis, which may exhibit regions such as head, body, and tail. Another commonly known pattern is that seen in the anuran amphibia, in which several vasa efferentia remove the sperm from the testis and deliver them into the longitudinal canal of Bidder in the wolffian body. Subsequent transfer is by way of renal collecting tubules and the wolffian duct. In the birds, on the other hand, the general pattern is much like that of mammals, with the exception that a rete as such does not exist, but the antrum testis, a thin-walled canal, may be its equivalent.

Alverdes ('26) recounted such facts as the above and pointed out that the architectural plan seen in the lizards does not conform to any of these schemes; his description based on *Lacerta* is confirmed in the same genus by Padoa ('33) and by my own findings in *E. fasciatus*. In this lizard pattern, septulae testis, tubuli recti, and a rete are lacking. Tubuli contorti converge to a testicular hilus where sperm are poured into a small space. A small testis outlet duct proceeds from this space, extends through the mesentery, and enters the epididymis where it connects with a longitudinal canal that gives rise to a number of vasa efferentia. These empty into the ductus epididymis which makes up the bulk of the organ, and which continues posteriorly as the vas deferens. Thus the term "epididymis," applied to a discrete organ, includes longitudinal canal, vasa efferentia, and the ductus epididymis, all supported by appropriate stroma or connective tissue, and enclosed in a capsule.

The testis of *E. fasciatus* is an ovoid organ, around 3.0×5.0 mm. in size. During the active season of spring it is relatively larger, a rich yellow in color, turgid, and about 0.03 to 0.05 gm. in weight. During the other seasons it is relatively smaller, more of a dirty white in color, somewhat flabby, and from 0.01 to 0.02 gm. in mean weight. Histologically it consists of convoluted seminiferous tubules enmeshed in a thin stromal network of supporting tissue, with interstices between tubules occupied in part by interstitial cells, capillaries, and other small blood vessels. Enclosed by the capsular tunica albuginea, the organ is supported in place by the mesorchium. From a point on the medio-dorsal part of the testis the very small testis-outlet duct arises. This duct extends through the mesentery to the epididymis.

The epididymis is a fusiform body, usually yellowish in color, the wider anterior end of which tapers abruptly while the posterior end narrows more gradually to the formation of the small vas deferens. The front end of the epididymis lies dorso-laterally from the testis. The small yellow adrenal gland, roughly one-fifth of the epididymis in length, lies near the anterior end of the epididymis and is inseparably bound up with the epididymal structures in the same capsular investment. A very short cubical epithelium, in which nuclear size is large relative to cell size, forms the lining of both testis outlet duct and the epididymal longitudinal canal. Careful examination of favorable material enables the tracing of the outlet duct from the testicular hilus, through the mesentery, and into the epididymis where it joins the longitudinal canal at about the middle of the latter. The longitudinal canal divides frequently and gives rise to drawn-out tips, some of which end blindly, but most of which give rise to vasa efferentia. A section-by-section study of two cases revealed eleven vasa efferentia so initiated in each. These efferent ducts are lined with cubical epithelium which has a very definite basal membrane and cells with large nuclei. Sections of these ducts occupy the anterior end of the epididymis, although a few extend far posteriorly.

The ductus epididymis originates by "head-on" junctions in which a vas efferens merely changes character and assumes the structural make-up of the epididymal duct; in one observed case two efferent ducts affected such a transformation simultaneously in forming the epididymal duct. Further along in its course the ductus epididymis receives the other vasa efferentia by "angular" junctions, the maximum number of such observed junctions is five. The ductus epididymis is not constant throughout its length; best generalized descriptive statements would include: (1) columnar epithelium height is generally greatest in the anterior end, least at the posterior end; (2) lumen diameters in general increase posteriorly, maximal diameter occurring in the posterior end; (3) tubule diameters are in the main constant, with a slight tendency for posterior increase.

As to the metanephric kidney of lizards, Krause ('21) gives a good description from the standpoint of general histology, based on *Lacerta viridis*, while embryological aspects of the kidney are discussed by Leydig (1872) and Braun (1877). Regaud and Policard ('03 a, b) described a sexually different preterminal tubule in serpents which was confirmed by Zarnik ('10). The latter author also described an initial collecting tubule in *Lacerta agilis* which he claimed was identical in the two sexes. This view was contradicted by Reiss ('23 a, b) who established in males of *L. agilis* a cyclic development of the kidney synchronous with that of the "interstitial gland." These "sexual segments," or sexually differentiated preterminal uriniferous tubules of the lizard kidney have also been described by Herlant ('33), Regamey ('35), Takewaki and Fukuda ('35), and they have been verified in *Eumeces fasciatus* as will be implemented below.

The dark red kidneys of *Eumeces fasciatus* lie far back in the body cavity in the usual retroperitoneal position, the two organs being separate anteriorly, united posteriorly. In the interior of the organ, large spaces in the center of the renal mass represent branches of the vein that collects blood from glomeruli and renal tubules, the vena renalis revehens. The

renal corpuscles are scattered at random in the interior in the general region of these venous branches; all elements of the nephron histology can be recognized although diligent search is necessary to find instances where the pavement epithelium of the capsule of Bowman transforms into the cubical epithelium of the initial uriniferous tubule. This initial tubule (order I of Krause, '26) is small, has granular cells and a small lumen. Such tubes are found concentrated peripherally on the ventral side, scattered in the interior, and concentrated dorsally not so near the periphery. The alignment suggests origin of such tubes from nephrons, extension ventrally where a convolution occurs, then a loop dorsally where the major convolution occurs. The next tubule in line (order II; Krause, '26) is smaller in diameter, has cells of non-granular cytoplasm, and is located principally near the dorsal side although a few are found centrally. Occurring all over the kidney, both collecting and outlet ducts converge ventrally and medially. Convergence of the latter near the middle of the kidney initiates the ureter which receives other outlet ducts as it proceeds posteriorly. The tubes of especial interest here are the "sexual segments," described as "preterminal segments" of the uriniferous canal, hence they include the collecting ducts and outlet ducts. Aside from position and relation, they are further characterized by being invested with a capsule of connective tissue and perhaps smooth muscle, whereas the rest of the kidney structure is merely suspended in the general renal stroma.

B. Seasonal variations in the reproductive tract

1. *January.* Testes examined in January show large seminiferous tubules which are turgid, have thick epithelia and well-developed lumina. In the organ as a whole there is little intertubular space; interstitial elements are inconspicuous as compared to tubular elements. The interstitial cells occur for the most part singly, although a few groups of four or five were to be seen in one testis. These cells are around 9.0 micra in diameter, have lightly stained cytoplasm, and possess nuclei

that are plump, round, and about 5.0 micra in diameter. Within the seminiferous tubules the nuclei of the Sertoli syncytium are uniformly more darkly stained than the syncytial cytoplasm. Flaky chromatin granules occur in the nuclei, and one or more nucleoli are in evidence as intense black dots. The Sertoli nuclei exhibit a variety of shapes which include oval, triangular, trapezoidal rectangular, etc. Greatest nuclear dimensions do not exceed 11.0 micra and the least do not go below 6.0 micra, and from thirteen to eighteen such nuclei occur per tubular cross section. Quantitative aspects (table 1) of these January testes give evidence of a possible correlation with fixation date in the case of tubular diameter. In one testis fixed January 11th the tubule diameter averages 159.9 micra whereas in three others fixed January 23rd, it averages over 200 micra, and in one case reaches 222 micra.

In the testicular germ line, the luminal one-third to one-half of the predominant cells of the epithelium are primary spermatocytes. While a few are at diakinesis, the majority are in stages of the leptonema, pachynema, or synapsis. In the earlier testis (January 11th) there is no suggestion of more advanced stages. In two of the later testes there is in addition to these predominant elements a thin luminal fringe of secondary spermatocytes at interkinesis (fig. 1), while in the fourth organ, a very few spermatids occur in addition. Most of the lumina contain masses of detritus made up of the same sort of material that lines the tubes themselves.

In the epididymis of January-fixed lizards the vasa efferentia are lined by high cubical epithelium the cells of which are ciliated and have round nuclei about 3.0 micra in diameter which contain scattered chromatin granules imparting a rather dark color. A very distinct basement membrane delimits the epithelium at its base. The tubules are around 28 micra in diameter, enclosing lumina with diameters of 12 to 15 micra. Posteriorly the larger ductus epididymis appears. The organ fixed later in the month (January 23rd) has larger epithelial height and tubule diameter (28 micra and 83 micra) than the organ fixed earlier (January 11th; 16 micra and 62 micra).

TABLE 1
Mean size relations and other aspects of primary and secondary sexual structures in *Eumeces fasciatus*
in various months of the year.¹

MONTH	JAN.	FEB.	MAR.	APRIL	MAY	JUNE	JULY	AUG.	OCT.	NOV.
<i>Testis</i>										
No. of cases	4	3	3	5	4	2	4	2	2	1
Sem. epith. ht.	55.9	70.2	48.9	70.9	56.4	45.9	37.3	32.3	53.5	61.7
Lumen diameter	61.4	77.3	70.0	87.3	73.5	51.7	27.6	20.4	30.1	66.5
Sem. tubule diam.	199.2	223.3	172.4	229.2	182.4	139.7	101.9	84.8	136.9	190.3
Relative no. of:										
Spermatogonia	4	4	4	4	4	4	1	1	1	1
Cytes 1	1	3	3	3	4	4	4	0	0	2
Cytes 2	4	2	2	2	3	3	0	0	0	0
Spermatis	4	1	1	1	1	1	0	0	0	0
Mature sperm	0	4	4	4	4	3	0	0	0	0
<i>Ductus epididymis</i>										
No. of cases	2	5	3	4	4	2	2	2	2	1
Epith. height	21.9	38.5	36.9	42.7	41.9	24.1	17.3	13.6	17.5	23.8
Lumen diameter	28.9	61.6	51.4	65.2	68.7	41.9	26.7	30.7	18.5	24.0
Tubule diam.	72.6	135.7	125.3	150.7	160.3	89.5	89.2	57.9	50.5	62.5
Secretion evidence	4	2	2	1	1	1	4	4	0	0
<i>Kidney "sexual segment"</i>										
No. of cases	2	3	3	3	2	1	2	1	2	1
Epith. height	17.8	31.2	27.3	31.1	35.6	21.5	12.1	27.7	11.4	16.7
Lumen diameter	11.9	22.7	20.1	17.9	24.5	20.9	10.8	20.6	20.6	10.7
Tubule diam.	46.5	82.9	74.4	69.0	95.9	64.1	34.2	68.7	41.1	42.1
Secretion granules	0	3	2	2	1	2	4	4	0	0

¹ Size relations of epithelial heights, lumen diameters, and tubule diameters are expressed in micron units. Germ cell types, evidences for secretion in the ductus epididymis, and secretory granules in the kidney are expressed on a scale of abundance in which the numbers mean: 1, very numerous, dominant; 2, large numbers but not dominant; 3, average to small numbers; 4, quite small numbers, scarce; 0, absent.

The epithelial cells are definitely columnar since their height (table 1) is around seven times their width (3 to 5 micra). Cellular cytoplasm is slightly granular, and the lumina are empty except for a few scattered strands of a material that may be secretion. Nuclei of the epithelial cells are mostly oval, 5.0 to 8.0 micra in greatest dimension, and tend to be basal in position within the cells.

While the epididymal ducts are large, they are not in the organ as a whole too compact, since the general stroma is abundant. Capsular tunics made up of straight or wavy lines, doubtless of connective tissue and smooth muscle, surround the ducts; tunics vary from 8.0 to 15.0 micra in thickness.

In the kidney of skinks fixed in January, a somewhat greater diameter differentiates the tubules of the "sexual segment" from the rest of the uriniferous canal in which the usual cubical cells are seen. The "sexual segment" epithelial cell height (table 1) is considerably greater than the width (2.0 to 7.0 micra). The round nuclei of the columnar cells are basal in position and about 5.0 micra in diameter. In one case the cellular cytoplasm is finely granular, while in the other slight granular concentrations occur near the apical surfaces or poles of the columnar cells.

2. *February.* Testicular Sertoli nuclei occur to the extent of 10 to 15 per tubule cross section, each nucleus 5.0 by 10.0 micra in mean dimension; interstitial cells are 9.0 by 12.0 micra in mean size and contain nuclei averaging 5.0 micra in diameter. Less similarity to the condition seen the previous month and more marked changes characterize other testis features, however: seminiferous tubule sizes are significantly greater (table 1), and marked changes have occurred in the germ line. Instead of constituting a fairly constant cellular wall around the tubule as it did in January, the distal or luminal portion is produced into a number of finger-like lobes around 41 micra in height which project into the lumen, in some cases nearly occluding it. These lobes arise from a basal, continuous or non-lobed, portion of the epithelium which is around 36 micra in thickness. The germ line exhibits a pre-

dominance of spermatids, with secondary spermatocytes next in abundance (fig. 2, table 1). The metamorphosis of spermatids into mature sperm involves a number of changes, and the very advanced stages, young non-tailed sperm, occupy the luminal borders of the lobes, from a very thin fringe to a definite layer in thickness. The general mass of the lobes is made up of less advanced spermatid stages, with very young ones forming a layer in the basal non-lobed portion of the epithelium. This basal layer is, however, in the main composed of secondary spermatocytes; also present are a few basal primary spermatocytes and a thin discontinuous layer of spermatogonia, a few of which show signs of mitotic activity.

In the epididymis the vasa efferentia are much like those seen in January, with tubules around 28 micra in diameter lined by a ciliated epithelium slightly higher (8 to 12 micra) than wide (5 micra) surrounding a lumen 8.0 to 11.0 micra in diameter. In a few sections of larger ducts with a higher epithelium no cilia are evident, but dim basal plates and granular cytoplasm give evidence of degenerate ciliation. The ductus epididymis exhibits a higher epithelium, larger lumen, and greater diameter (table 1) in February-fixed animals than in those of the previous month. Cell heights are four times cell widths (7 micra); round nuclei about 4.6 micra in diameter are definitely basal in position. In the epididymis as a whole, the stromal material is less conspicuously exhibited, and the tubule capsules are thinner (5 to 8 micra) than in the cases studied for the previous month.

Other innovations in these epididymal ducts include the presence of sperm. Although in no cases abundant, sperm are present in the lumina of all tubes observed, the number varying from very rare to rather appreciable amounts. Furthermore, the ducts give evidence of active secretion: granules in the cell cytoplasm in varying amounts, but present in all cases; strands of stained material, presumably secretion, extending from the luminal cell surfaces, present in three cases; presence of a "brush border" of small projections (semi-circular, rod-like, or knob-like) from the apical surfaces of the

cells, present in two cases; and presumed luminal secretion, a mass of non-cellular amorphous material in the lumen that takes the stain in apparent proportion to density or amount present, observed in four cases.

In the kidney the "sexual segment" evinces a mean size increase over the preceding month (table 1), and separate consideration of three cases studied shows that epithelial height and tubule diameter both increase progressively in specimens fixed, respectively, on the eighth, nineteenth, and twenty-second of the month. In the first, only a fine granulation appears in the cellular cytoplasm, in the second kidney, the presence of large secretory granules is marked, and there is a little secretion in the lumen. In the third kidney the granules are abundant. The cells of the segment are columnar, about seven times their width (3 to 5 micra) in height (table 1), and their apical halves contain the secretory granules.

3. *March.* Mean quantitative data recorded in table 1 are adversely affected by one specimen which proved to be somewhat juvenile. In the other two testes studied the epithelial heights are 65 and 50 micra, lumen diameters, 96 and 82 micra, tubule diameters 240 and 182 micra. Thus these attributes in the March material are slightly less, but of the same general magnitude as the cases studied for the previous month. From nine to fourteen Sertoli nuclei are found per tubule, in size approximately the same (10.0 by 6.0 micra) as those seen in February. Interstitial cells averaging 17 by 7 micra have nuclei 5.0 micra in diameter which are plump and round in shape.

Germ line elements are slightly more advanced than in February. Although still not numerous, more sperm occur in the lumina of the seminiferous tubules. The lobed epithelium is dominated by still later stages of spermatid development which may be regarded as young sperm. In spite of the dominance of transforming sperm and spermatids of younger stages, there occur secondary spermatocytes, primary spermatocytes in pachytene and synaptic stages, and the thin discontinuous basal layer of spermatogonia.

In the epididymis the vasa efferentia are much like those studied in the previous month with respect to epithelial height (6.0 to 11.0 micra), lumen diameter (15 micra), and tubule diameter (35 micra). A few such tubes which originate from the longitudinal canal are larger with taller epithelium (12 to 17 micra) and show evidence of ciliary and basal granule degeneration. In the ductus epididymis the size relations are similar to those seen in February (table 1). Sperm are present in the duct lumina, and epithelial secretory activity is evident in a "brush border," in secretion strands, and in the presence of a mass in the lumina.

The "sexual segment" of the kidney is easily recognized by size difference. One specimen has a mean tubule diameter of 106 micra which exceeds any studied so far, although the juvenile specimen affects the means recorded in table 1 in an unfavorable manner. The cells of the epithelium are definitely columnar with heights (table 1) several times their widths (3 to 9 micra). The most striking feature of these cases is the abundance of the distinctive secretory granules which occupy practically the entire cell length. Some secretion material occurs in the lumina.

4. *April.* Testes exhibit ten to nineteen Sertoli nuclei per tubule cross section existing in the same sizes and varied shapes as seen previously. Interstitial cells average 8 to 16 micra in greater dimension with plump round nuclei about 6.0 micra in diameter. In the seminiferous tubules table 1 will show that maximal development in size of elements is reached during this month. Individual tubule diameters range as high as 276 micra, epithelial heights as high as 89 micra, lumen diameters as high as 96 micra. In the germ line, however, no great differences are noted, the lobulated seminiferous epithelium still being dominated by transforming sperm and other spermatid stages. Maturing sperm vary from a thin fringe one or two cells deep to a deep layer ten to twelve cells in thickness with about one-third the epithelial thickness or six cells as the average. Proximal to this occur secondary spermatocytes at interkinesis and a few primary spermato-

cytes at pachytene or synapsis. In one specimen a general retardation is evident in that the epithelium is made up largely of secondary spermatocytes with fewer primary ones. At the lumen there is a very thin fringe of transforming sperm. At some points in these April cases the spermatogonial layer is mitotically active.

Epididymal vasa efferentia are quite comparable to those previously described. Larger ducts (59 to 62 micra) have higher epithelial cells (16 to 24 micra) while smaller ducts (25 to 44 micra) have epithelial cells 7 to 10 micra high with cilia and plate-like basal granules. Lumina are much the same (8 to 28 micra). The ductus epididymis (table 1) has increased in size, having tall columnar cells about one-sixth as wide with oval nuclei definitely basal in position. The epithelium is secretory and sperm are present in the tubular lumina. The tubes of the ductus epididymis are surrounded by a capsule averaging 6.0 micra in thickness and composed of 6 to 12 wavy fibers concentrically arranged. However, the size of the tubules themselves renders somewhat inconspicuous the capsular and general stromal material.

In the kidney the state of the "sexual segment" remains much like that of the preceding month. Size relations are slightly less, the lumina contain a small amount of stringy secretion material, and the cellular cytoplasm contains the distinctive secretory granules in the distal one-third to one-half of the cell.

5. *May*. Testes studied for this month reveal two new aspects indicating a change in the trend of events: (1) regression in size of tubular components (table 1); (2) less compact cells and exposure of Sertoli syncytium both indicate incipient depletion of germinal material. In developmental state, however, the germinal elements present an advance over the previous month. In general, sperm are present in the tubule lumina, tailed sperm adhere to the luminal fringe of the Sertoli and the epithelium is dominated by very advanced spermatids or transforming sperm practically exclusively. Other components of the epithelium include small numbers of

younger spermatid stages, secondary spermatocytes half of which are at interkinesis and half in early divisional stages, largely prophases, a few primary spermatocytes, and the spermatogonia. In one case (no. 62), very long lobes populated by an average of thirty-four transforming sperm each reduce the non-lobed portion of the epithelium to a basal layer no more than two cells thick in some places; in another testis (no. 5) lobes are less numerous and each is less densely populated by cells; and a third case (no. 59) is unique in that the epithelium is not lobed, and perhaps correlated with this, an unusual amount of Sertoli cytoplasm is exposed. In these May testes from ten to twenty-one Sertoli nuclei occur per tubule, each nucleus being around 6 by 10 micra in dimension. Interstitial cells measure about 7 by 14 micra and have plump round nuclei about 5 micra in diameter.

The epididymal duct of these lizards reaches maximal size during the month of May (table 1). The large plump tubules render the epididymal stroma and tubule capsules quite inconspicuous (fig. 3). The capsules are quite thin (3 to 6 micra). Luminal masses, strands, and a "brush border" attest to epithelial secretory activity, and the tube lumina contain sperm. The vasa efferentia are essentially similar to those seen in April.

In the kidney the "sexual segment" also reaches during this month a peak of development in both size of tubular components and secretory state as judged by the presence of numerous distinctive secretory granules (fig. 5). This aspect is most highly developed in one case (no. 62) where the cells of the "sexual segment" tubules, literally gorged with these particles, dwarf the other aspects of the renal structure.

6. June. During this month testicular components show further size decreases (table 1). The state of the germinal material in one of the cases studied (no. 253) is much like that seen in May: sperm in the lumen, sperm adhering to the luminal fringe of the Sertoli, and very advanced spermatid stages or transforming sperm dominating the epithelium, in some cases extending back to the basal layer of spermatogonia. Only

in rare instances are secondary or primary spermatocytes seen. Sertoli nuclei average fourteen per tubule, and the basal spermatogonia exhibit considerable divisional activity. The other case studied for this month is much more extreme. The tubules are not crowded, intertubular spaces are much larger than previously observed, and small lumina are present in only about half the tubes. In those tubules with lumina restricted or absent, their position is indicated by a light space where the Sertoli cytoplasm extends beyond the epithelial cells and occludes the lumen. The germ cells are not numerous, and are mostly spermatogonia at rest although a few basal ones are in mitosis.

In the ductus epididymis of no. 253 the sizes of tubular components are only slightly smaller than those of the previous month, there is abundant evidence of secretory activity, and the lumen contains sperm. The vasa efferentia are quite similar to those studied previously as to diameter, epithelial height, and lumen diameter. The cells are ciliated and have basal plates. The presence of sperm in these tubes shows that sperm are still being ejected by the testis. The other case studied for this month (no. 54) shows no sperm in either vasa efferentia or ductus epididymis, no evidence of secretion in the latter, and reduced size which depresses the mean record for the month in table 1. The epididymis of this case is more typical of the month to follow.

In the "sexual segment" of the kidney regression in size (table 1) is paralleled by a recession in the number of secretory granules in the cell of the tubules; these tubules, however, remain much larger than other parts of the uriniferous canal.

7. July. By the month of July, testicular tubular components have regressed approximately 100% in size as compared with the maximum observed in April (table 1). The small tubules are lined by spermatogonia, largely non-mitotic, which form a layer not generally exceeding six cells in thickness, sometimes as thin as three cells. The cells are loosely arranged, exposing the Sertoli. While a few lumina persist, the lumen has in general been obliterated by projections of

Sertoli cytoplasm which extend beyond the distal limits of the thin germinal layer and occlude the luminal space. A peculiar feature of these testes is a wrinkling of the capsules surrounding the tubules in such a manner as to produce invaginations partially dividing the tubules into segments. One is lead to postulate a general contraction of testis volume which squeezes the contents thus forcing an approximation of epithelial linings, obliteration of the lumen, and a buckling of the capsules thus producing the folds which appear as partial tubular septa.

In the epididymis the aspect is quite different from that of previously studied cases. Size relations of the ductus epididymis are smaller (table 1), the oval nuclei of the epithelial cells are not basal in position, and there is little evidence of secretory activity save in the presence of some material in the lumina, which may be residual secretion produced earlier, but not yet extruded. Small numbers of sperm occur in the lumina. In general, stromal material and capsules (9 to 21 micra in thickness) are conspicuous at the expense of tubular material. In the vasa efferentia epithelial height (8.0 micra), lumen diameter (12 micra), and tubule diameter (28 micra) do not differ markedly from previously described organs.

The kidney "sexual segments" have regressed by both size decrease (table 1) and the practical disappearance of secretory granules. In these tubules for the first time identification must depend in part on characters other than greater size, and chief among such characters is the capsule, which is 3 to 6 micra thick.

8. *August.* The testis attains the most involute condition during this month (table 1) with small seminiferous tubule diameters, thin epithelia, and lumina non-existent with few exceptions. Tubule capsules are thicker (0.6 to 1.2 micra) than seen previously. The Sertoli cytoplasm is quite evident, a matrix in which the germ cells lie fairly loosely, although somewhat more compactly than in July. Sertoli cytoplasm extends beyond the epithelium and occludes the luminal space.

Sertoli nuclei, around twenty to the tube, lie against the basement membrane.

The seminiferous epithelium consists very largely of spermatogonia lying two to six cells deep and falling into two size groups: (1) larger ones of which diameters are 13 to 16 micra with nuclei 7 to 9 micra in diameter; and (2) much more abundant smaller ones 8 to 11 micra in diameter with nuclei around 6.5 micra in diameter. Mitotic activity of these stem cells of the germ line is much more in evidence than it was during July; a mean of 11.5 cells were in mitosis in five true cross sections populated by an average of 73.5 cells each. The predominant phase was prophase with the spireme still enclosed in the nuclear membrane. The situation just described obtains in both testes studied for August with the exception that in one, no. 25, there occur a few primary spermatocytes some of which are in synapsis.

The ductus epididymis is also quite involute, with decreased size relations (table 1), thick tubular capsules (6 to 17 micra), non-basal nuclei, and, in one of the cases studied, no evidence of secretion except residual luminal masses, while in the other case a few knobs forming a "brush border" occur. General stromal and capsular tissue is conspicuous. In the vasa efferentia size relations are similar to those previously described, and cilia are present but not as conspicuously as formerly.

Only one kidney was studied for August. While size relations are somewhat greater and a few secretory granules still persist, an involute condition prevails (table 1).

9. October. By the month of October the testes reflect a measure of resumed activity. Fairly marked increases in quantitative relations (table 1) are seen, due to a greater number of germ cells, a higher epithelium, and more closely compacted cells. While a few lumina exist, they have not been generally reconstituted. The seminiferous epithelium is composed in the main of spermatogonia most of which are at rest, although small isolated spots of mitotic activity are seen. The spermatogonia are again referable to two size

groups: one of more numerous and smaller cells 7 to 10 micra in diameter with 5- to 7-micron nuclei, and one of larger cells 11 to 13 micra in diameter with 7-micron nuclei. Both are found undergoing mitosis. These spermatogonia also exhibit two nuclear forms: the "crust-like" type has scattered chromatin aggregated into clumps which naturally appear quite dark, while the "dust-like" type has the chromatin more diffusely scattered into fine dust-like alignment on the linin network. These nuclear types can not be correlated with nuclear sizes.

The ductus epididymis is in complete involution; size relations are small (table 1), there is no evidence of secretion, stromal material is prominent, and capsules are thick (9 to 20 micra). Lumina may contain residual secretion in small amounts mixed with cellular debris.

The same picture of involution appears in the "sexual segment" of the kidney, which is not the least differentiated by size of secretory granules. The cells of the segment average only 11.4 micra in height, and are around 6 micra in width, hence are low columnar in form. No granules occur in the cells, which are, in fact, clearer than other parts of the renal tubules. Capsules are around 3 micra thick.

10. November. By this month continued testicular activity has produced size relations comparable to those of January (table 1); the increased tubule diameter is accompanied by the reconstitution of the lumen and a higher epithelium populated more thickly by more closely compacted cells. Predominant cells are spermatogonia, both crust-like and dust-like nuclear types being in evidence, as well as the large and the more numerous small-sized cells. Whereas such mitoses as have been observed during the past 3 months have occurred basally, fairly marked activity is now seen along the luminal border. The cells taking part are around 8 micra in diameter with mitotic figures slightly over 4 micra in diameter. These cells are apparently young spermatocytes, and the activity being manifested as maturation activity. A few cases of the pachytene stage are in evidence.

In the epididymis dormancy still prevails with epididymal duct sizes still small, no evidence of secretion, and the stromal and capsular material prominent (fig. 4). Vasa efferentia are much the same as before.

The "sexual segment" of the kidney remains small in size with secretory granules extremely rare (fig. 6).

C. Summary of the normal cycle

From January onward the testis of *E. fasciatus* exhibits a gradual increase in seminiferous tubule diameter, lumen diameter, and epithelial height which leads to maximal size relations in April. Paralleling this, dominance of the epithelium by primary spermatocytes in January shifts to young spermatids by February, and to late-stage spermatids by March. These older germ line elements maintain dominance through April, May, and June, and the very few mature sperm produced in February assume typical numbers in March, become maximal in April, and taper off during May and June accompanied by a general depletion of all germinal elements and reduced size relations. Final exhaustion of germinal material of the current season, in late June or early July, reduces the seminiferous epithelium to a thin layer of spermatogonia from two to six cells in thickness, a layer that has been built up at a slow but consistent rate, beginning with sporadic spermatogonial mitoses as early as February. The sparse germ-cell population leaves the exposed Sertoli extending distally toward the lumen, and loss of tubular turgidity, aided probably by the elasticity of the tunica albuginea, results in readjustments which cause tubular crumpling and obliteration of the lumen. These adjustments reach conclusion in August, the month of minimal sizes and of greatest involution. Meanwhile spermatogonial mitoses are slowed up in July, accelerated in August and October, and by November the lumen has been reconstituted and sizes approximating those of January restored. Germ cells in November are largely spermatogonia, but a few primary spermato-

cytes are present, and the fact that some have reached, but none passed, the pachytene indicate that it is a stable condition in which the cells may remain till the onset of the next cycle.

In the accessory reproductive organs, active secretion is begun by the ductus epididymis in February, about co-incident with the production of the first sperm in the testis. Increases in both secretory power and size of tubular components result in a maximum in May, a month later than the testicular maximum. Although evidence of secretion persists as late as July, regression follows with attainment by August of complete involution which remains till the beginning of the next cycle. In the duct lumina, very small numbers of sperm appear in February, maximal numbers in April, reduced numbers thereafter till July, after which they do not occur. In the "sexual segment" of the kidney a size differentiation is evident as early as January. This difference increases during ensuing months and reaches a peak in May. Exhibition of the distinctive secretory granules begins about the middle of February and also reaches a peak in May. Involution follows with minimal size being attained in July, while a very few secretory granules may persist as late as August.

Functional state of the organs concerned, as indicated by histological state, tends to place the "breeding period" in the months of April, May and June. This is confirmed by observation of lizard overt activity, in that courtships have been observed to occur during the latter half of April and in May and June, while actual copulations have been observed on the following dates: April 25th, April 28th, May 14th, May 27th, May 30th, and July 3rd.

Other aspects of the organs discussed above do not exhibit any marked seasonal variations. Sertoli nuclei are variable in number at all times, the range being from 9.5 to 21.2 per tubule. In the epididymis, diameters of the lumina and tubules of the vasa efferentia are somewhat larger in April and May, but no other seasonal effects are to be noted.

EXPERIMENTAL STUDIES

The foregoing account of reproductive changes in the normal *Eumeces fasciatus* affords a point of departure for further studies, as well as a background for evaluation and interpretation of such studies. Extension of the analysis includes gonad removal and replacement therapy utilizing testosterone propionate ("Oretone," Schering) and estradiol benzoate ("Progynon B," Schering). Lack of a large supply of animals was a most serious hindrance, and the results are offered as indicative or preliminary, not as conclusive ones.

A. The effect of castration

In table 2 are summarized some of the observations on seven male lizards gonadectomized and then sacrificed after varying periods of castrate life.

Because the secondary sex organs of comparable control animals are in a high state of development, particular interest attaches to consideration of three animals sacrificed on April 27th, May 2nd, and July 2nd, after castrate periods of 459, 630, and 64 days respectively (fig. 9). Epididymal duct epithelial height, lumen diameter, and tubule diameter are typically involute. Although very slight, there is evidence of a degree of secretory activity on the part of the epithelium. Epididymal stroma is very much in evidence, investing tunics are thick (9 to 13 micra), epithelial cell nuclei are not basal. Controls on the other hand have insignificant stroma, thin capsules (5 micra) and basal nuclei. Epithelial cells of the castrates are not only shorter (table 2) but also narrower (4 micra) than those of controls (6 to 8 micra). The "sexual segment" of the kidney is also quite involute; sizes contrast with those of controls to the extent of at least 100% (table 2), and there is complete absence of secretory granules in contrast to their marked presence in controls (fig. 8).

Three additional castrates (nos. 173, 211, 123) were fixed during the month of July. In quantitative characters, lack of secretion, stromal and capsular prominence, and nuclear posi-

TABLE 2

Mean results of castration and of hormonal injection on the ductus epididymis and the "sexual segment" of the kidney in male *Eumeces fasciatus*.¹

ANIMAL NO.	DAYS CASTR.	SACR. DATE	DUCTUS EPIDIDYMIS				"SEXUAL SEGMENT"			
			Epith. ht.	Lumen diam.	Tubule diam.	Sec.	Epith. ht.	Lumen diam.	Tubule diam.	Sec.
Castrates										
126	459	4-27	12.1	11.6	35.8	4	12.0	15.2	12.3	0
Cn.	...	...	42.7	65.2	150.7	1	31.1	17.9	69.0	2
92	630	5-2	12.9	24.0	50.7	4	10.9	14.0	35.6	0
Cn.	...	...	41.9	68.7	160.3	1	35.6	24.5	95.9	1
17	64	7-2	15.4	11.9	42.4	0	10.9	13.9	36.3	0
Cn.	...	...	32.9	60.8	125.3	2	21.1	20.9	64.1	2
173	27	7-13	15.8	19.6	51.1	0	10.7	12.3	33.7	0
211	47	7-13	10.7	25.1	47.6	0	11.6	16.2	39.1	0
123	184	7-27	12.2	8.4	32.7	0	12.7	12.1	37.4	0
Cn.	...	...	17.3	26.7	89.2	3	12.1	10.8	34.2	4
63	7	8-12	21.2	27.9	75.3	0				..
Cn.	...	...	13.6	30.7	57.9	0				..
Injected with testosterone propionate										
74 (normal)			53.5	73.1	179.9	1	52.1	26.3	131.4	1
Control			23.8	24.0	62.5	0	16.9	10.7	42.0	0
Difference			124.0%	204.6%	187.9%		208.7%	143.3%	214.4%	
193 (unilat. castr.)			48.8	93.9	191.5	1	46.5	21.0	114.1	1
Control			13.6	30.7	57.9	4	27.7	20.6	68.7	4
Difference			258.7%	205.9%	230.7%		68.7%	2.0%	66.0%	
252, 179 (normals)			36.4	73.4	145.0	3	51.1	20.6	117.0	1
Control			17.3	26.7	89.2	4	12.1	10.8	34.2	4
Difference			110.4%	174.9%	62.5%		322.3%	91.0%	242.2%	
238, 260 (bilateral castr.)			37.0	52.6	126.6	3			113.6	..
Controls			17.3	26.7	89.2	4	12.1	10.8	34.2	4
Difference			113.9%	97.0%	41.9%				222.1%	
Injected with estradiol benzoate										
172, 236 (normals)			27.0	30.5	83.9	3	10.5	15.5	36.5	0
Controls			17.3	26.7	89.2	4	12.1	10.8	34.2	4
Difference			56.1%	14.2%	-5.9%		-13.6%	43.8%	6.7%	
254, 115 (bilateral castr.)			29.4	41.8	99.9	3	11.3	14.2	36.6	0
Control			17.3	26.7	89.2	4	12.1	10.8	34.2	4
Difference			69.9%	56.6%	11.9%		-6.4%	31.8%	7.1%	

¹ Mean measurements are expressed in micron units; differences are expressed as percentages of the control values, and are positive unless indicated to be negative; secretory state is indicated on a scale described for table 1.

Abbreviations: Castr., castrate; Cn., control; Cns., controls; Epith., epithelium; Diam., diameter; Sacr., sacrifice; Sec., secretory state; Ht., height; unilat., unilateral.

tion, epididymal duct structures are quite similar to those of the height-of-season cases just described although castrate-control differences are less since the controls are themselves in early seasonal involution. Epithelial heights do not deviate by as much as 3 micra from the mean of 12.9 micra which compares favorably with 13.5 micra for the height-of-season cases. Tubule diameters of 51, 48, and 33 micra show a decrease which may be considered proportional to the 27-, 47-, and 184-day castrate periods; furthermore, the latter exhibits the smallest lumen. In the kidney, the "sexual segment" evinces no secretory granules contrasting with a few persistent ones in July controls, and there is a slight size difference.

The castrate series includes a seventh case which was sacrificed, only 1 week after castration, on August 12th, a date when normal animals are themselves at seasonal involution; hence its similarity to the controls.

Separate consideration of these castrates reveals that a castrate period of 27 days produces perceptible regressive changes, a 47-day period shows such changes well advanced, while at 64 days the condition approaches the involute state seen in long-time castrates of 6 months or more.

Since epididymal vasa efferentia undergo little change in the normal seasonal cycle, it is not surprising to find them little affected by castration save in that ciliation was slightly less prominent. Castration further appeared to reduce the intensity of the red coloring of the head, and castrates were never observed to court or copulate although caged with adult females in season.

B. The effect of androgen administration

With respect to control of the reproductive processes of *Eumeces fasciatus* the analysis has thus far revealed (1) that early activity of the epididymis and kidney in February is preceded by some testicular activity; (2) the testis attains an activity maximum about 1 month before the epididymis and kidney (May), and starts regression (June) about 1 month before they do; (3) the months from July to January are

characterized by relatively little testis activity and none on the part of the accessory organs; (4) absence of the testis causes the accessories to lapse into an involute state. Evidence from study of both normal and experimental animals thus strongly suggests the testis as the source of the agent necessary for development and maintenance of the active state in the accessory organs.

As a further step in the analysis androgen (testosterone propionate) was administered to both castrate and normal lizards. For the sake of brevity certain details as to treatment of the six animals are omitted from table 2. Animal no. 193, beginning 11 days after right unilateral castration received 0.5 mg. of testosterone propionate daily for 20 days, a total dosage of 10.0 mg., and was killed and fixed on August 25th. Animal no. 74, a normal animal, received twenty injections of 0.1 mg. each plus a twenty-first injection of 0.09 mg., making a total dosage of 2.09 mg., after which sacrifice occurred on November 14th. Animal no. 252 received 0.7 mg. of the androgen in seven equal injections over 12 days with autopsy on July 8th, while no. 179 received in nine equal injections 0.9 mg. over 21 days with sacrifice and autopsy on July 17th. As to the castrates, beginning 31 days after bilateral castration no. 238 was given eight injections of 0.1 mg. each over 14 days, while no. 260 received eight injections of 0.1 mg. each over 15 days, the first being administered 6 days after bilateral castration. Respective autopsy dates were July 10th and July 11th.

The three smaller injected animals (nos. 252, 238, 260) died, apparently as a result of the injections, and it may be that the dosage employed was excessive since larger animals withstood the treatment. Practically constant observation during the experimental period was practiced and autopsy was thus accomplished at death or very shortly after. Observable symptoms that preceded death were general sluggishness, inability to use the hind limbs well, and light, apparently labored, rather infrequent breathing movements.

1. *Effects on the testis.* Testes of injected animals sacrificed in July (nos. 179, 252) do not differ significantly in weight from control testes. Seminiferous tubule diameters average 88.0 micra as against 89.2 in controls, epithelial heights are 78% that of controls, and lumen diameters are 89% as great as in controls. Lumina are in reality rare, most of them being occluded as is normal at this season. Other typically involute aspects of these testes include invaginated tubule capsules, visible Sertoli cytoplasm, and loosely arranged germ cells; germ cells are even less compact than in control normals, there being an average of twenty-four cells per tubule in the injected cases as against an average of forty-eight in the controls.

The residual left testis of the injected unilateral castrate (no. 193) presents a picture very comparable to that of a normal of comparable fixation date (August 25th): tubule diameters approximately the same, lumina scarce, Sertoli cytoplasm exposed; however, the seminiferous epithelium of the injected testis is about two cells thick forming an average of twenty-five cells per tubule, whereas in controls it is from two to six cells deep and forms about seventy-three cells per tubule.

Greater differences are seen in the case of no. 74, fixed in November where testis weight is 0.07% of body weight as compared to 0.21% in controls. Seminiferous epithelium height, lumen diameter, and tubule diameter are respectively 48%, 46%, and 67.6% as great as in controls. Formation of primary spermatocytes up to the pachytene is in progress to approximately the same degree in both injected and control animals. While controls have at this season a recently repopulated epithelium, reconstituted lumen, and fairly compact germ cells, this injected testis has a fairly loosely populated epithelium, lumina not reconstituted or at best not uniformly, fairly conspicuous Sertoli. In fact, this testis is typical of the most typical involute normal condition seen in August except for the degree of spermatogenetic activity.

The general trend of the testis responses to androgen appears to be regressive. In July, more extreme reduction of the lumen, less compact germ cells, and fewer cells per tubule present a situation to be expected if the testis adjustments of this period were accelerated while the slow repopulation of the epithelium were decelerated or even halted. In August, the month of normal maximum involution, the principal difference between injected and normal animals is the smaller number of germ cells per tubule in the former. In November, a period of testicular activity revival, seminiferous tubules, lumina, and epithelia are markedly smaller than in controls although spermatogenetic processes in single cells have occurred to an approximate normal extent.

2. *Effects on the epididymis.* Fresh weights taken at autopsy give a general indication of epididymal response to androgen. The mean weight of 11.6 mg. for November normal organs may be compared with that of 29.2 mg. for the four epididymides from injected animal nos. 193 and 74. The four organs from no. 252 and 179 average 30.6 mg. as against 10.0 mg. for July normals. Injections thus produce epididymal weights greater than those of normal animals by 100 to 200%. The four epididymides from the two injected castrates (nos. 260, 238) averaged 13.2 mg., which is only a little greater than the normal control weight at 10.1 mg.

Internally, quantitative aspects of ductus epididymis make-up are in nos. 193 and 74 more than 200% greater than in controls. Granular cytoplasm, evidences of secretory activity, round nuclei basal in position, columnar cells, and thin tubule capsules mark these organs as similar to those of normals at the height of seasonal activity. The same is true of the injected normal animals sacrificed in July (nos. 252, 179), where a less marked hypertrophy, from 63% to 175% greater than controls, is evident, and in the injected castrates where sizes are 42% to 113% greater than in controls (table 2).

3. *Effects on the "sexual segment" of the kidney.* In the late-season injected normal males (nos. 193, 74) "sexual segment" tubular elements are from 65% to 200% greater

than in controls. Epithelial cells are columnar, their heights (table 2) being six to eight times their widths; nuclei are round and basal. There is some secretion in the lumina, and the cellular cytoplasm exhibits granules occupying at least the distal two-thirds of the cell-length (fig. 7). Of the normal injected animals sacrificed in July, one (no. 179) presents an aspect very similar to that just described, with abundant secretion granules in the cells, some luminal secretion, basal nuclei, and cells just under 6 micra in width and about eight times as high (table 2), and with generally hypertrophied tubular elements. The other animal sacrificed at approximately the same time (no. 252) actually died and there is evidence of damage to the renal epithelium. Neither the epithelium nor the lumina could be studied, but measurement of the internal diameters of the capsular investments of the tubules gave an approximation to the tubule diameters; such data indicate a diameter similar to that of no. 179.

The two injected castrates (nos. 238, 260) also give indication of damage to the renal epithelium, especially that of the preterminal segments. Cells lying in clumps or masses with no discrete lumen or epithelium lie in spaces surrounded by the investing capsules. Evidence adduced from capsule measurement indicate a diameter comparable to that of uninjured kidneys from injected animals, with a stimulation amounting to an increase of 232% compared to normal controls.

C. The effect of estrogen administration

Results of preliminary experiments on the effect of estradiol benzoate on accessory male characters are presented. Normal male no. 236 received 25 RU (rat units) in five daily and equal injections, and no. 172, a total of 15 RU in three equal and daily injections, with tissue fixation occurring on June 29th and July 4th, respectively. Castrate lizard no. 254, beginning 4 days after bilateral gonadectomy, received eight injections of 5 RU each and a ninth of 3 RU over a period of 12 days; castrate no. 115, beginning 31 days after bilateral gonad-

ectomy, received 43 RU in the same manner over a 12-day period. These castrates were autopsied and the tissues fixed on July 6th. In all four estrogen-injected cases death terminated the experiment, autopsy occurring a few moments after death. Summarized data are presented in table 2.

Mean weight of the four testes from the injected normal animals is 7.3 mg., somewhat less than the mean of 11.7 mg. for comparable controls. The seminiferous epithelium is 30 micra thick as compared to 37 micra in controls, and the loosely-arranged germ cells average twenty-five per tubule which is less than in controls.

Epididymal weights of the four organs from injected normals average 5.1 mg., those from injected castrates, 10.1 mg., as compared to the mean of 10.0 mg. for comparable controls. Moderate secretory activity is exhibited by the normals, less by the castrates. In quantitative aspects, the ductus epididymis of castrates responded more than did the normals (table 2). While not uniformly so, nuclei tend to be basal, round in shape although a few are oval, and the capsules around the ducts are relatively thick.

In the kidney the "sexual segment" shows no trace of secretory granules in either injected normals or injected castrates; in both the epithelium is slightly reduced, and the tubule diameter very slightly increased, as compared to controls (table 2), and capsules around the tubules are thin.

D. Summary of experimental studies

Attention has already been directed to the fact that the normal seasonal and sequential behavior of the epididymis and kidney of *E. fasciatus* is suggestive of testicular control. Castration and injection experiments designed to test such hormonal control give powerful support to the argument, as may be emphasized in concise form by use of the following tabular summary of results in which normal animals sacrificed during January, June, July, August, October, and November

have been arbitrarily designated as "at involution," while those sacrificed during February, March, April, and May are designated "at activity."

DUCTUS EPIDIDYMIS	EPITH. HT.	LUMEN DIAM.	TUBULE DIAM.
Mean of all normals at involution	19.7	28.5	50.4
Mean of all castrates	15.3	18.4	47.9
Mean of all normals at activity	40.0	61.7	143.0
Mean of all androgen-injected liz.	43.9	73.3	160.8
SEXUAL SEGMENT			
Mean of all normals at involution	17.9	15.9	49.5
Mean of all castrates	11.4	13.9	32.5
Mean of all normals at activity	31.3	21.3	80.6
Mean of all androgen-injected liz.	49.9	22.6	119.0

Thus castrates, even if sacrificed in the season of greatest normal activity, exhibit epididymides and kidneys fully as involute as those of normal animals at the depth of seasonal regression; trends measured quantitatively are paralleled by the degree of secretory activity manifested. On the other hand androgen-injected animals, even if sacrificed at the depth of normal involution, may exhibit the general size, hypertrophied tubular relations, cellular detail, and secretory state of the normal active season; furthermore, the androgen can prevent or repair the changes following castration in both kidney and epididymis.

On the testis itself, the results indicate that androgen administration causes a measure of inhibition, an effect to be expected on the basis of the Moore-Price hypothesis of endocrine interaction (Moore and Price, '32; Moore, '35). Thus the inhibitory effect of excess androgen on the pituitary produces an accentuation of normal testis regression in July and an inhibited state in November when a measure of revived activity is seen in normal testes.

Results of estradiol benzoate injection indicate that both epididymis and kidney are sensitive to the estrogen, but the response is erratic and does not have the definiteness, the consistency, or the amplitude of that produced by testosterone propionate.

DISCUSSION

In view of Herlant's ('33) fine paper, no review of the literature in the historical sense has been attempted here. However, because of the small number of papers specifically devoted to general and endocrine physiology of reproduction in lizards, an attempt is made below to correlate all pertinent items of information.

A. The normal reproductive cycle

In short and summary papers, Reiss ('23) has summarized the testicular cycle in *Lacerta muralis* and *L. agilis*, and Kehl ('35) has discussed the cyclic phenomena of several reptiles of the Sahara desert. A much more extensive paper by Daleq ('21) reports on the cycle in *Anguis fragilis* in which the testis is dominated by primary spermatocytes, commonly at pachytene, till as late as March, with secondary spermatocytes next in abundance. By early April the seminiferous tubule lumen is cleared and dominance has shifted to spermatids and young sperm, with transformation into sperm completed by the end of the month. In May most of the sperm are evacuated, spermatogonial divisions begin, and a few primary spermatocytes may appear and advance as far as maturation. In the main, however, spermatogonial divisions continue through June and July leading to tubule re-population and to occlusion of the lumina by August. At this time the basal-luminal sequence of germ cells is: large occasional spermatogonia, young primary spermatocytes, synaptic spermatocytes, pachytene spermatocytes, and finally a few rare first maturation divisions. By November a few spermatids may be present but in the main maturation is halted at the pachytene. Herlant ('33) confirms this outline of germ line behavior in *A. fragilis* and adds that during February (primary spermatocyte dominance) the seminiferous tubule diameter is 180–200 micra, that it increases to the maximum of 240 micra in April, recedes to 120–150 micra with sperm evacuation in May, then increases to 190 micra in June and 200 micra by November.

Comparison of this cycle with that of the *E. fasciatus* testis reveals that in the latter tubule size changes are of greater amplitude, the period of sperm production is longer, sperm evacuation is less rapid, spermatogonial divisions begin earlier and continue longer, primary spermatocytes appear later, and spermatid dominance and sperm production begin earlier. In both animals testis activity maximum occurs in April, active germ cells of two annual cycles co-exist for a time, and the pachytene appears to be a stable condition of some duration during the winter. In general, the various processes of the *Eumeces* testis occur at slower rates and over longer periods than do those of *Anguis*.

Another form studied by Herlant ('33), *Lacerta muralis*, agrees more closely with *E. fasciatus* in that primary spermatocytes dominate the seminiferous epithelium in January, spermatids in February, and spermatids and transforming sperm through March, April, and May; furthermore, sperm are gone by July. Primary spermatocytes appear earlier in *L. muralis*, however, since they are dominant by August, and substantial numbers of spermatids are present before testis repose is attained. Regamey ('35) gives data on a third European species, *Lacerta agilis*. Like *E. fasciatus* and *L. muralis*, spermatogenetic activity is in full progress by March, with tubules 190 micra in diameter exhibiting stages from synapsis and pachytene to metamorphosing sperm. The next generation of germ cells dominate the epithelium in *L. agilis* by June, and by July these have largely reached the pachytene and established an epithelium 220 micra thick. A significant layer of spermatids is present by September; some of these form sperm which degenerate and the epithelium falls to 190 micra which it maintains through quiescence.

The above summarizes the findings reported in the most thorough and detailed studies on lizard reproductive periodicity. On American lizards some data has accompanied experimental reports. Thus in *Anolis carolinensis* Evans ('35) describes the winter or quiescent condition (October 30th to April 4th) of the testis as one with seminiferous tubules and

lumina of 80 micra and 35 micra respectively with an epithelium of 12 micra populated by spermatogonia with a sprinkling of spermatids. Turner ('35) states that in *Eumeces laticeps* breeding begins in May with seminiferous tubule diameters of 330 micra which decrease to 160 micra during regression in the fall; during winter involution they have an inconspicuous obstructed lumen, conspicuous Sertoli, and loose syncytium.

Turning now to the epididymis, the epididymal duct epithelium of *L. muralis* (Herlant, '33) is 18-20 micra high in January, and it reaches 50 micra in April, a height reached a month earlier by *A. fragilis* from an initial 30-micron height in February. In both, the epithelium begins secreting in March, a month later than in *E. fasciatus*. In *A. fragilis* and *E. fasciatus* an epididymal activity peak is reached in May, and in all three epididymal regression is in progress in July and is completed by August. Padoa ('33), also reporting on *L. muralis*, places the secretory period from the end of March to the end of June, with maximal epithelial height at 70 micra which falls to 15 micra at extreme involution. In *L. agilis* Regamey ('35) observed epididymal ducts 85-100 micra in diameter with epithelia 30 micra high in March with only slight secretory activity evident. By June secretory maximum had been passed and the tubule had receded to 18-20 micra, indicating a short active period. Repose is reached in July and maintained till the next cycle. In *Takydromus tachydromoides* Takewaki and Fukuda ('35) observed epididymal duct epithelial heights of 50-65 micra in April, with a maximal height of 65 micra and active secretion persisting from the middle of May to the beginning of August. Following repression and quiescence during August, a short period of activity recurs from the middle of September to the middle of November. In *Anolis carolinensis* (Evans, '35) data are available for only the involute period during which an epithelium 8-10 micra high lines epididymal ducts 30-35 micra in diameter. Turner ('35) found no indication of secretion in

Eumeces laticeps, during the involute period, in ducts 18–42 micra in diameter with epithelia 11 micra high.

The “sexual segment” of the *E. fasciatus* kidney exhibits size differentiation as early as January, with secretory granules appearing in February. In *Anguis fragilis* size differences are slight in February and not till the latter part of April are the tubules enlarged and full of granules (Herlant, '33). In both *A. fragilis* and *E. fasciatus* maximal size and secretory activity are seen in May, with regression evident by the following month. In *Lacerta muralis* (Herlant, '33) the segment reaches full activity with cells 30–80 micra high in April, a condition maintained through May after which regression occurs with cells receding to 18 micra by July. In *L. agilis* (Regamey, '35) tubes 50–60 micra in diameter are lined with epithelial cells 22 micra high in spring, secretory activity is maximal in June, but has ceased by July. *Takydromus tachydromoides* exhibits thick segments 25–40 micra in diameter in April with numerous secretory granules which persist through May, June, and July. Though the cells remain high, granules are not seen from the end of August to the middle of September when a short spurt of activity occurs from late September to mid-November (Takewaki and Fukuda, '35).

In general, those lizards that have been studied in some detail reveal a fundamental pattern in cyclic reproductive processes. Within this pattern minor variations include differences in specific time of initiation and termination, differences in speed and in duration of specific functions of organs or their parts. In general one major active period per year is exhibited, the principal variation being the short autumnal spurt of activity in *Takydromus tachydromoides*. *Eumeces fasciatus* conforms to the general pattern as is indicated by the phenomena described above.

Daleq ('21) reported a multiplication of the Sertoli nuclei of *Anguis fragilis* in April coincident with spermiogenesis and the beginning of spermatogonial proliferation, a phenomenon which he linked with growth of syncytial cytoplasm and in-

creased requirements imposed on the nutrient portion of the testis. However, Harlant ('33) made careful enumerations on Sertoli nuclei in *A. fragilis* and established means which revealed that such cyclic augmentation of these nuclei was very slight. No such fluctuations were observed in the Sertoli nuclei of *Eumeces fasciatus*.

The testicular interstitial cells of *A. fragilis* undergo a cyclic behavior based on variations in number, volume, and inclusions (Herlant, '33), all of which increase during the early months of the year. The maximal conditions maintained through May are followed by regressive changes. Essentially similar cycles were observed in *Lacerta agilis* from April to June (Regamey, '35) and in *Lacerta muralis* with a maximum in May (Herlant, '33). In the latter form the interstitial cells were difficult to observe, and special methods were necessary. In *Eumeces fasciatus*, consideration of these elements of the testis has been postponed to this point because these cells are not conspicuous, and they are difficult to observe, especially after the month of June. A typical interstitial cell is one of variable shape (round, oval, triangular, trapezoidal, etc.), with greatest dimension around 12 micra, least dimension around 7 micra, and a nucleus about 5 micra in diameter. While no particular variations in number are observed, there is suggestive data of a maximum in March and April with respect to size.

B. Experimental approach

The classic initial approach to determine possible testicular control of accessory reproductive organs, castration, was applied to lizard material by Matthey ('29) who observed castrate male *Lacerta agilis* for as long as 1 year. In the castrates the normally sexually dimorphic femoral glands assumed and remained in an essentially female condition, and the distinctive green male coloration failed to develop. Absence of reliable external indicators such as these in *E. fasciatus* relegates determination of castration effects to

study of internal features where marked effects are described above.

As to the time required for appearance of castration effects, lack of material prevented the presentation of critical data on *Eumeces fasciatus*, but it was indicated that such changes are definitely perceptible at 27 days after the operation. In *Takydromus tachydromoides*, Takewaki and Fukuda ('35) found: 15 days after castration, essential normality; 22 days, reduced ductus epididymis epithelial height and decreased secretory granules in the "sexual segment"; 30 days, no secretory activity and large capsules in the epididymis and no secretory granules in the "sexual segment"; 41 to 75 days, the epididymis at complete repose, "sexual segment" nearly so. *Dalmation Lacerta serpa* are also essentially normal at 15 days post-castration, but at 22 days slight "sexual segment" regression is noted, at 6 weeks or 2 months, they are typically involute, and specimens sacrificed in April after 95 days of castrate life resemble the December involute condition (Herlant, '33). *Lacerta muralis* exhibited evident effects 15 days after castration, and was in a state comparable to winter quiescence at 30 days, and was even more involute after 2 or 3 months (Padoa, '33). Long-time castration effects are reported by Regamey ('35) on *L. agilis* sacrificed in March about a year following castration in which the epididymis exhibited epithelial heights of 12 micra as against 30 micra in controls, the tubules 55 micra in diameter as against 90 to 100 micra in controls. Herlant ('33) reports that *L. serpa* castrated in May and sacrificed the following October differed little from the involute controls, and Regamey ('35) reports that castrate *L. agilis* males do not differ much from controls in July or September.

Substantial unanimity of all published reports thus favors the thesis that the development and maintenance of the hypertrophied active state of the lizard accessory reproductive organs is dependent on the testis. The chief absolute castration changes in both the ductus epididymis and kidney "sexual segment" are loss of secretory power and reduction in size

of tubular components. The generalization is indicated that such changes appear from 15 to 30 days after gonadectomy, going well marked after the latter period. Castration-induced regression and normal seasonal regression result in the same involute state, the former process being somewhat more rapid as Regamey ('35) has pointed out.

To determine whether or not testis hormone action operates under the "all or none law" or on a proportional basis, Padoa ('33) utilized unilateral castration of *Lacerta muralis* and obtained a slight reduction of ductus epididymis lumen diameter; subtotal castration yielded more decisive results in that a condition of accessory organs intermediate between the normal and total castrate was observed. At autopsy Regamey ('35) discovered that a supposed totally castrate *L. muralis* was in reality subtotal, a small piece of the testis having regenerated and formed a small knot about 1/200 of the volume of a normal testis. The epididymis and "sexual segment" were in active secretion, but other indicators were lacking (femoral gland development, color, etc.). It is thus indicated that the accessory sex organs respond to the hormone in a proportional manner, but additional evidence is necessary before this can be accepted as conclusive.

A slight but noticeable degree of secretory activity in an otherwise involute epididymis is described above in long-time castrates of *E. fasciatus* sacrificed at the seasonally active period. Since the spring season is one of greater alertness, activity, and organic well-being for the lizard in general, such secretory activity is probably a reflection of the generally enhanced metabolic rate and is quite unrelated to the reproductive system as such. A similar case of such a "metabolic effect" is noted in castrate *L. agilis* by Regamey ('35).

Testis grafting, as a converse to castration, was employed by Herlant ('33) who placed testis tissue in the coelomic cavity of female *L. serpa* in an effort to induce the typical male "sexual segment" picture. Although they could be recovered as small bits of tissue as late as a year later, the grafts failed to implant and become vascularized, and the

kidneys remained unaffected. Essentially similar experiences are reported by Matthey ('29) and Regamey ('35). Neeser ('40) succeeded in getting two "takes" out of four implants of testis of *L. viridis* into the highly vascular liver, although no effect was noted in the femoral glands as indicators of hormone activity. In a repetition, grafts of testis into the liver followed by sixteen subcutaneous implantations of pituitary gland over 38 days caused the femoral glands of castrates to begin secreting on the eighth day and to soon attain the typically active state. The clarity of these results is vitiated by the subsequent finding that pituitary alone has a limited capacity to provoke femoral gland secretion. The most successful grafting experiments are those of Takewaki and Fukuda ('35) who report graft recovery from six out of seven castrate animals; the grafts contained hypertrophied interstitial cells, degenerating germ line elements, and a few recognizable persistent germ cell stages. In four of the seven cases kidneys were normal with the "sexual segment" in activity, in three cases there was less marked activity. While epididymal ducts were no larger than in castrate controls, the epithelium was actively secreting. These results thus yield fragmentary evidence that the testis, even in a foreign site, tends to alleviate the effects of castration. Since it appears that lizard tissue is rather refractory to testis grafting, this type of replacement therapy does not afford conclusive information.

Greater success has followed the administration of androgenic substances by injection. Thus, where Herlant ('33) failed with testis grafts, and where Regamey ('35) failed, not only with grafts, but also with injections of testis extracts, Kehl ('38) succeeded in causing the kidney of female *Uromastix acanthinurus* to develop the full active state of the male "sexual segment" by injection of benzoate of androsterone. Introduction of testosterone propionate into normal and gonadectomized *Anolis carolinensis* maintains the epididymis and vas deferens in activity in males and develops the "sexual segment" in both sexes (Noble and Greenberg, '40). The 61% of which was absorbed over 6½ weeks from 2.8 mg.

to 6.5 mg. pellets of testosterone implanted into *Sceloporus* caused an added hypertrophy of the ductus epididymis but no great effect on the "sexual segment" (Forbes, '40, '41). In *Sceloporus occidentalis*, testosterone propionate produced a 25% increase in ductus epididymis diameter, and an epithelial height of 35 micra contrasted with 11 micra in controls. This epithelial height increase approximating 200% is similar to that reported above in *E. fasciatus*, while the diameter increase of 25% is less than the 41% found in *Eumeces*.

On the testis itself, androgen injection was indicated as somewhat inhibitory in *E. fasciatus*; however, Forbes ('40, '41) reports that testosterone stimulated spermatogenesis in *Sceloporus* sacrificed in late March (breeding period for *S. undulatus floridanus*, Newman and Patterson, '09). Conclusions on this subject must await more extensive data.

Estrogen administration does not elicit responses of great clarity or consistency. Administration of theelin produces a 40% increase in epididymal weight in *Eumeces laticeps* (Turner, '35) and a 12% weight increase in the vas deferens of *Anolis carolinensis* (Clapp, '37). On the other hand, Noble and Greenberg ('40) failed to get a response from either epididymis or "sexual segment" of *A. carolinensis* from injection of estradiol dipropionate, and Forbes ('40) reports testis atrophy, reduced spermatogenesis, smaller epididymal tubules, and no kidney response resulting from implantation of oestrone pellets into *Sceloporus*. Administration of theelin to *Sceloporus occidentalis* results in a depression of ductus epididymis epithelial height to 6 micra as compared to a control height of 11 micra (Gorbman, '39). Recorded above are results of estradiol benzoate injection into *E. fasciatus* which result in moderate stimulation of the epididymal duct, and in the "sexual segment" no secretory activity and decreased epithelial height accompanied by increased diameter of lumina and tubules.

From the above review it is evident that the much clearer and more consistent evidence from androgen administration into lizards produces results that agree with the more frag-

mentary evidence from testis grafting and which are the converse of those following castration, so far as accessory reproduction organs are concerned. Conflicting indications and lack of clarity characterize androgenic effects on the testis and estrogenic effects in general.

SUMMARY

Thirty-six normal male *Eumeces fasciatus* were utilized for seasonal study of the testis, epididymis, and "sexual segment" of the kidney to establish the reproductive cycle. Preliminary experimental results, based on seventeen males, are also presented. All results are discussed in terms of other reports on lacertilians in the literature. Early seasonal increase in seminiferous epithelial heights and in diameters of lumina and tubules results in maximum size in April followed by regression reaching complete involution by August. Late seasonal revival of activity results by November in size of testicular elements comparable to those seen in January. Primary spermatocytes predominate in the germinal epithelium in January, secondary spermatocytes and spermatids in February, with spermatids and metamorphosing sperm dominating from March until late June when the germinal material of the current season is exhausted. Mature sperm appear in February and typical numbers are produced as long thereafter as germinal material is available. Spermatogonial proliferation begins in April and continues slowly until the epithelium is repopulated in November, at which time some primary spermatocytes are present. Interstitial cells exhibit maximal sizes in March and April. Tubular elements of the ductus epididymis and "sexual segment" of the kidney exhibit early seasonal size increase. Secretory activity begins in February and reaches a maximum in May, coincident with attainment of maximal size. Ensuing involution becomes complete in August. Following castration of active males, the accessory organs recede to an essentially involute condition. Administration of testosterone propionate to castrate or inactive males stimulates both epididymis and kidney which

develop a size and secretory state comparable to that of normal animals at the height of seasonal activity. Suggestive testis inhibition was noted. Less marked and less consistent stimulative effects are noted in the epididymis and kidney following injection of estradiol benzoate.

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PLATE 1

EXPLANATION OF FIGURES

All figures photomicrographs, $\times 225$.

1 Portion of right testis of animal no. 127, fixed January 23, 1938. A thick seminiferous epithelium lines turgid, large tubules. Predominant primary spermatocytes occur basally, some secondary spermatocytes are near the lumen.

2 Portion of section of testis of no. 99, fixed on February 19th. Large tubules, capacious lumen, and a thick epithelium are exhibited, the latter produced into lobes. Metamorphosing sperm and spermatids occur near lumen, secondary spermatocytes in middle layer, and primary spermatocytes basally.

3 Portion of section of epididymis of *E. fasciatus* no. 62, fixed May 26, 1937, at height of activity. Lumen contains secretion and embedded sperm mass. Strands are seen, knobs are shown indistinctly. A thin capsule invests the ductus epididymis which has tall epithelial cells and basal nuclei.

4 Epididymis of no. 73, fixed on November 14th, in the involute period. Characteristics include small tubules, short epithelial cells, lack of secretion, non-basal nuclei, thick capsules and general abundance of stroma.

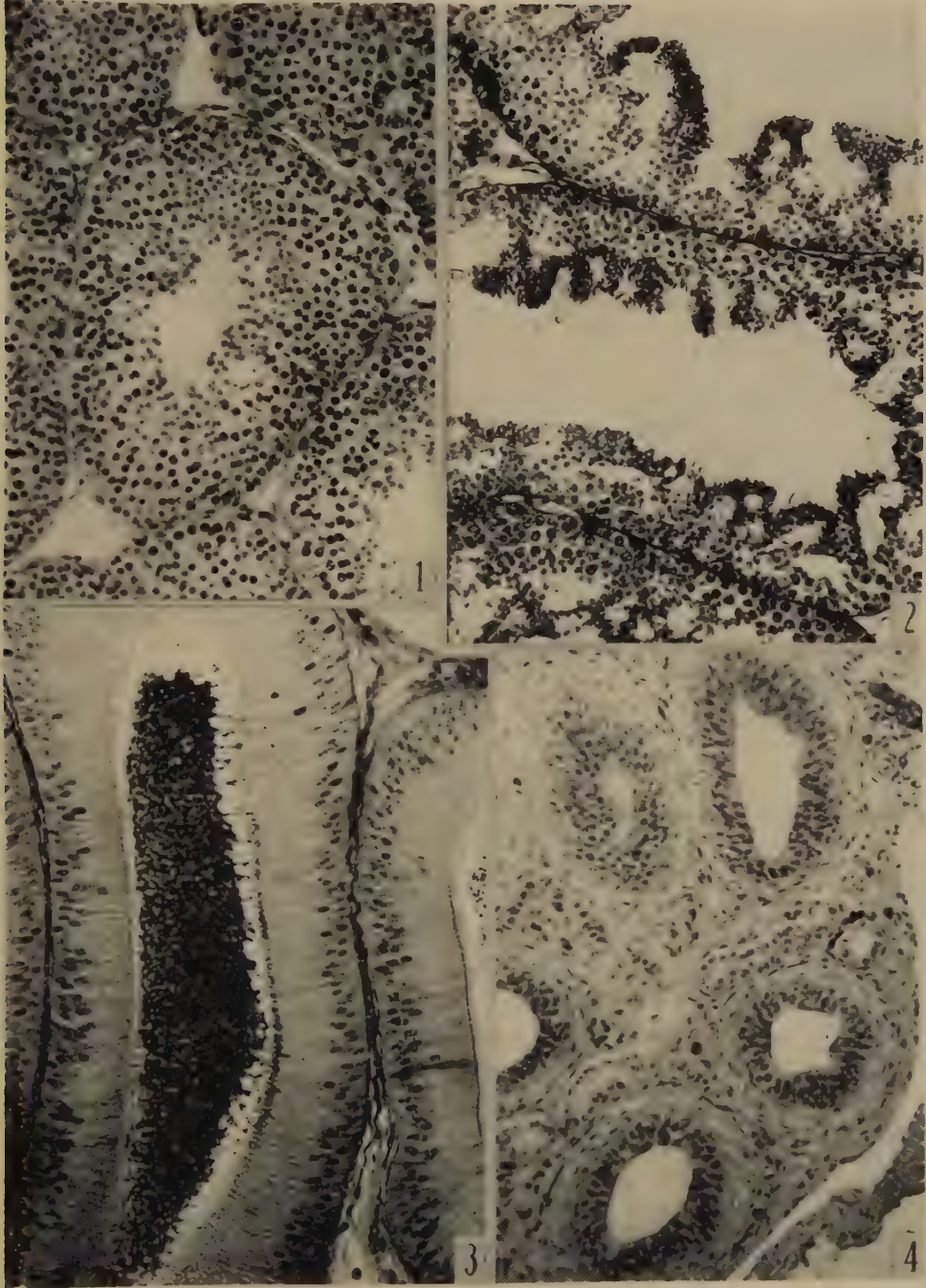


PLATE 2

EXPLANATION OF FIGURES

All figures photomicrographs, $\times 225$.

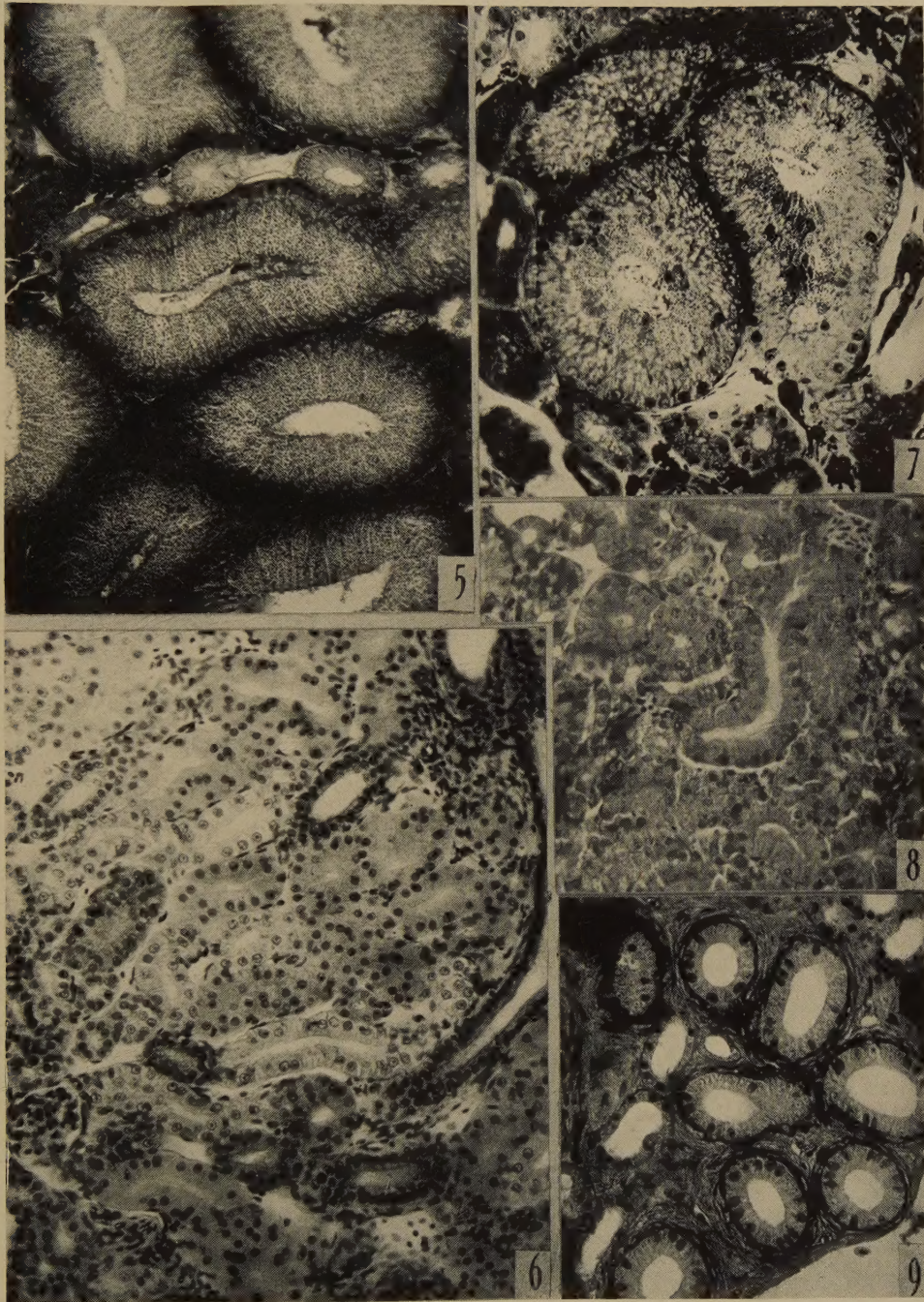
5 Portion of sectioned kidney of no. 62, a skink fixed on May 26th at height of activity. In comparison with other renal tubes shown, note large size, tall columnar cells, and secretory granules of the "sexual segment."

6 Portion of sectioned kidney of no. 73, fixed on November 14th during involute season. Encapsuled "sexual segment" tubules (with dark nuclei) are not differentiated in size, have short cells, and lack granules.

7 Portion of a sectioned kidney of no. 193, a unilaterally castrate skink that received 10.0 mg. of testosterone propionate, with fixation on August 25th, a season of normal involution. "Sexual segment" tubules are enlarged and granules are present. Compare with figure 5, contrast with figure 6.

8 Portion of kidney of no. 92, a 630-day castrate fixed on May 2nd. Note involute condition similar to that of figure 6.

9 Portion of sectioned epididymis of no. 92. Note the small ductus epididymis and its general similarity to the condition seen in figure 4.



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